EFFECT OF LOW MOLECULAR WEIGHT ORGANIC COMPOUND SODIUM 2-(2-HYDROXYPHENOXY)ACETYL)-L-PROLINATE ON RAT GASTRIC SECRETION STIMULATED BY CARBACHOL

To investigate the effects of low molecular organic compound (LMOC) on gastric acid secretion (GAS), pepsin output and the activity of H⁺,K⁺-ATPase in the conditions of carbachol stimulation. GAS stimulated by carbachol was measured by method of isolated perfused stomach, pepsin output was analyzed by colorimetric method for 120 minutes and the activity of H⁺,K⁺-ATPase was assessed spectrophotometrically by the level of anorganic phosphate on 20 white laboratory rats. GAS, pepsin output and the H⁺,K⁺-ATPase activity were decreased under the carbachol stimulation after injection of LMOC. LMOC decreases the gastric acid and pepsin secretion in terms of carbachol stimulation. The inhibition of H⁺,K⁺-ATPase activity is considered as mechanism of antisecretory property of LMOC.

Keywords: low molecular weight organic compound, stimulated secretion, hydrochloride acid, pepsin, H⁺,K⁺-ATPase.

UDK 577.15

Ya. Raetska, PhD, O. Morgaienko, PhD, T. Ischuk, PhD student, L. Ostapchenko, Doctor of sciences
Taras Shevchenko National University of Kyiv, Kyiv, Ukraine

AMINOTRANSFERASE ACTIVITY IN RAT BLOOD SERUM UNDER MALIGNANT GUERIN'S CARCINOMA GROWTH UPON TREATMENT WITH ANTIOXIDANT DRUG "GRIN"

The malignant growth of Guerin's carcinoma was shown to induce increase of aminotransferases' enzymatic activities. It was established that introduction of the antioxidant substance "GRIN" led to normalization of aminotransferases' activities in blood serum and also had essential inhibitory effect on the tumor growth.

Key words: Guerin's carcinoma, aminotransferases, an antioxidant.

Introduction. Annually there are nearly 6 million new registered cases of malignant disease all over the world. Meanwhile, uterine cancer (adenocarcinoma) is a widespread disease among women ranking 4th place after breast, skin, and gastrointestinal cancers.

The rate of uterine cancer incidence increases steadily. In Ukraine, this value is 26.4 per 100,000 population. Women are starting to get sick at the age of 40-54 years old, the peak of the disease occurs at the age of 60-64 years [1,2]. At the present time Guerin's carcinoma (GC) is known to be an optimal model of this pathology for research. GC is a strain of modified cancer derived from the rat spontaneous adenocarcinoma.

Nowadays natural substances are proved by experimental and clinical studies to be promising in combination therapy of cancer. Drugs increasing antitumor resistance, preventing metastasis development and cancer recurrence, attenuating chemotherapy toxicity are of particular interest. So use of drugs that have ability to normalize physiological antioxidant system and increase resistance to malignization is important for oncology [3, 4].

Biochemical parameters of animal and human organisms are known to change under tumor growth [5]. So the aim of the research was to reproduce experimental model of adenocarcinoma and to determine activities of aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) in rat blood serum under tumor growth in condition of studied substance "GRIN" administration.

Blood serum activities of hepatic enzymes AST and ALT are known to be informative characteristics of pathological process because the liver plays a major role in metabolism and is involved into dysfunction of an organism under tumor growth. Herewith AST and ALT values indicate the effect of test substances under malignization [6,7].

Materials and methods. The experiments were conducted on 130 female white rats with weight 180 ± 20 g, which were kept on the standard vitamin diet. GC obtained from donor rats was inoculated to the animals by subcutaneous injection (20% suspension of tumor cells in 0.9% NaCl) in rat hindlimb [8]. Some animals had been treated daily with the studied substance at various doses for 23 days after tumor transplantation. The animals from control group were administered with 1 ml of 0.9% NaCl. At the 7th day after inoculation tumor volume had been measured for period till 25th day. The animals were decapitated under light ether anaesthesia on the 23rd day after tumor transplantation.

To obtain rat blood serum peripheral blood was kept in thermostat for 40 min at 28°C and then underwent centrifugation at 2500g for 30 min [9].

Enzymatic activities were measured using the biochemical analyzer "Humalyzer 3000" with kits for colorimetric determination of AST and ALT activities and were expressed as equivalent units per liter (EU/L) [10].

Statistical analysis was performed using software for statistical analysis in Microsoft Excel. Unpaired Student's t-test was used to compare values of different groups, and P values < 0.05 were considered to be significant [4].

Results and discussion. Treatment with drugs normalizing physiological antioxidant system and increasing resistance to malignization is important in medicine and represents one of the directions of complex oncology research [11, 12]. The substance "GRIN" designed by "World grinization system" company (Ukraine) is a powder form of the purified protein fractions from the Far-Eastern Holothurian and the worm culture made by special multistage, non-enzymatic, low temperature technology, that allows to avoid denaturation of proteins, to increase their bioavailability, and to retain their globular state with preservation of nuclear DNA's regulatory peptides and their functional properties.

The first step of investigation was determining effect of the substance "GRIN" on tumor growth in rats with inoculated CG. The following results were obtained (Figure 1). We found out that studied substance stabilized growth of GC. Such effect was observed predominantly at 18th and 23rd days after tumor inoculation in compare to GC control. Average tumor volume was increased steadily reaching the peak meaning at the 23rd day. Administration with "GRIN" in dose 200 mg/kg led to tumor volume decrease on 39.1% in compare to tumor control (rats with GC).
Comparing the dose effects of the substance "GRIN" on rats with inoculated GC we concluded that the most effective dose in our experiments was 200 mg/kg. The conclusion was based on 2 criteria: index of tumor growth inhibition and prolonging of lifespan in compare to control values.

Obtained results demonstrate expediency to research biochemical mechanisms of the substance's effect. Blood biochemical parameters are an integral characteristic responsible for condition of an organism. Particularly aminotransferase's activities are informative characteristics.

Data presented reveal increase of AST activity in blood serum of rats with GC about 5-fold at the 15th and 18th days after tumor inoculation (from 37.1 ± 0.12 to 200.6 ± 0.6* EU/L, and from 35.12 ± 0.2 to 163.7 ± 0.5* EU/L, respectively), and about 3-fold (from 31.1 ± 0.1 to 109.1 ± 0.1* EU/L) at the 23rd day compared to control. Meanwhile AST activity in blood serum of rats with GC under "GRIN" treatment was shown to change in a dose-dependent manner. The doses 100 mg/kg and 200 mg/kg caused gradual decline of AST activity at 18th and 23rd days after tumor inoculation comparing to rats with GC, while they were approaching the normal values.

In groups of rats administered with the substance "GRIN" in doses 100 mg/kg and 200 mg/kg AST, activity was decreased about 2-fold at the 23rd day after tumor inoculation in compare to GC group (from 109.1 ± 0.1 to 59.2 ± 0.3* EU/L, and from 109.1 ± 0.1 to 53.1 ± 0.1* EU/L, respectively) that may indicate functional recovery of liver, heart and skeletal muscles.

ALT determination in rat blood serum under tumor growth revealed following changes (Figure 3).
ALT activity in blood serum of rats with GC was increased about 3-fold at 15th and 18th days after tumor inoculation (from 45.1 ± 3.13 to 137.1 ± 0.1 EU/L, and from 44.3 ± 3.1 to 126.7 ± 0.3 EU/L, respectively), and 2-fold (from 42.1 ± 2.1 to 91.2 ± 0.1* EU/L) at the 23rd day in compare to control group meanings. Administration with the studied substance "GRIN" in different doses (10 mg/kg, 100 mg/kg, 200 mg/kg) led to gradual changes of ALT activity. The doses 100 mg/kg and 200 mg/kg caused 1.5-fold decrease of ALT activity in blood serum at the 23rd day after tumor inoculation in compare to rats with GC approaching to physiological normal value (from 91.2 ± 0.1 to 56.8 ± 0.6*, and from 91.2 ± 0.1 to 49.2 ± 0.9*, respectively). Stabilization of studied parameters in range close to normal values was found, and differences between groups were significant (P < 0.05). Essential changes in AST and ALT activities were observed. In the case of rats with GC administered with the substance "GRIN" in dose 200 mg/kg, significant attenuation of ALT and AST activities were observed (P<0.01) in compare to tumor control (rats with GC). We demonstrated the substance "GRIN" inhibited tumor growth. Such effect was observed predominantly at 18th and 23rd days after GC inoculation. So, it was established that malignant growth led to augmentation of AST and ALT activities in blood serum. Meanwhile administration of the studied substance in doses 100 mg/kg and 200 mg/kg resulted in attenuation of investigated enzymatic activities with tendency to approach the normal values. It occurred at the background of tumor growth inhibition.

We suggest that changes observed had occurred as a result of positive effect of the substance "GRIN" on blood flow organs and microcirculation of the internal organs and on prevention of catabolic reactions accompanying cancerogenesis. Decrease of AST and ALT activities is considered to follow the liver function recovery. It can be realized by mechanisms involving inhibition of oxidative reactions, stabilization of mitochondria functions, restore of hepatocyte membrane structures by such compounds of the natural substance "GRIN" as amino acids, vitamins, peptides [13].

Conclusions. Decrease of GC average volume in rats under administration with "GRIN" in different doses in dynamics was established. Obtained results indicate the dose 200 mg/kg as the most effective. An administration of this dose substance led to about 3-fold GC growth inhibition. AST and ALT activities were shown to increase in rat blood serum under GC growth. Administration with the natural substance "GRIN" led to decline of AST and ALT activities in blood serum of rats with GC. The dose 200 mg/kg was determined to be the most efficient approaching the enzymatic activities to control values.

References
УДК: 57.017.642:636.52/58:615.9

Віктор Макарович Білоус

Вступ. На сьогоднішній день в усьому світі зростає увага до розвитку нанотехнологій. Одними з найперспективніших є волокнисті вуглецеві наноматеріали. Враховуючи, що обсяги їх виробництва постійно розширяється, і надалі очікується тісний контакт людини та інших біологічних об’єктів із наночастинками, включення потенційних ризиків їх використання виявляється в якості одного з першочергових завдань [1]. Аналогічна ситуація, проте з природним волокнистим матеріалом – азбестом, спостерігалась в минулому у зв’язку з його активним впровадженням у будівництво інших сферах людської діяльності. Ентузіазм світової спільності в порівнянні з активністю волокна демонструє досить високий рівень. Так цікавим є те, що у більшості випадків ці ризики не відображаються всіма речами. Проте, багато вибірків, що відходять від більш відомих, повинні бути ретельно дослідженими.

Дослідження з використанням наноматеріалів зазначає, що використання біологічних ембріонів в дослідженнях погано впливає на фізіологічні процеси в організмі. На цьому стає ясно, що використання біологічних ембріонів в дослідженнях повинні бути зосереджені на розробці екологічно чистих методів. Тому є важливою задача розробки екологічно чистих методів використання наноматеріалів.

Основні методи. Експеримент здійснено на курячих ембріонах, ікубації яких велась на різних термінах. Усі ембріони були введені до різних термінів, зокрема на 19-й день і на 35-й день. Ембріони були введені до різних термінів, зокрема на 19-й день і на 35-й день.

Висновки. Отримані дані свідчать про те, що використання біологічних ембріонів без використання наноматеріалів може призвести до негативних наслідків. Тому є важливими розробка екологічно чистих методів використання наноматеріалів, що можуть бути використані в наукових дослідженнях.